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Colour change and camouflage in the horned ghost crab *Ocypode ceratophthalmus*

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Species that change colour present an ideal opportunity to study the control and tuning of camouflage with regards to the background. However, most research on colour-pattern change and camouflage has been undertaken with species that rapidly alter appearance (in seconds), despite the fact that most species change appearance over longer time periods (e.g. minutes, hours, or days). We investigated whether individuals of the horned ghost crab (*Ocypode ceratophthalmus*) from Singapore can change colour, when this occurs, and how it influences camouflage. Individuals showed a clear daily rhythm of colour change, becoming lighter during the day and darker at night, and this significantly improved their camouflage to the sand substrate upon which they live. Individuals did not change colour when put into dark conditions, but they did become brighter when placed on a white versus a black substrate. Our findings show that ghost crabs have a circadian rhythm of colour change mediating camouflage, which is fine-tuned by adaptation to the background brightness. These types of colour change can enable individuals to achieve effective camouflage under a range of environmental conditions, substrates, and time periods, and may be widespread in other species. *Biological Journal of the Linnean Society*, 2013, **109**, 257–270.

ADDITIONAL KEYWORDS: camouflage – circadian rhythm – colour change – crabs – predation.

INTRODUCTION

Anti-predator coloration is common in animals and crucial to survival in individuals of many species (Ruxton, Sherratt & Speed, 2004). Probably the most widespread type is camouflage, where an object resembles the background in order to prevent detection or recognition by an observer (Stevens & Merilaita, 2009). Although historically an important area of study and a key example of natural selection (Wallace, 1889; Poulton, 1890; Cott, 1940; Cook *et al*., 2012), research into camouflage has been particularly strong in recent years, receiving attention from biologists, psychologists, computer scientists, and art historians (reviewed by Stevens & Merilaita, 2009, 2011). Studies have investigated the types of camouflage that exist (e.g. Cuthill *et al*., 2005; Merilaita & Lind, 2005; Schaefer & Stobbe, 2006; Stevens *et al*., 2006; Fraser *et al*., 2007; Rowland *et al*., 2008; Skelhorn *et al*., 2010; Stevens *et al*., 2011), the adaptive value of camouflage (e.g. Vignieri, Larson & Hoekstra, 2010; Cook *et al*., 2012), how it works and relates to visual perception (e.g. Stevens & Cuthill, 2006; Troscianko *et al*., 2009; Zylinski, Osorio & Shohet, 2009), the role of sampling scale and ecological factors (e.g. Caro, 2009; Todd *et al*., 2012), and the molecular basis of adaptation (e.g. Nachman, Hoekstra & D'Agostino, 2003; Rosenblum *et al*., 2010; Manceau *et al*., 2011; Nunes *et al*., 2011). Despite this, most research investigating camouflage function has involved artificial systems, including artificial prey (e.g. Cuthill *et al*., 2005; Schaefer & Stobbe, 2006; Stevens *et al*., 2012), laboratory and computer studies (e.g. Merilaita & Lind, 2005; Fraser *et al*., 2007), and theoretical modelling (e.g. Merilaita, Tuomi &

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Jormalainen, 1999; Houston, Stevens & Cuthill, 2007). A major gap, however, is work studying and objectively quantifying camouflage in real species against the natural environment in which individuals are found (but see for example Merilaita, 1998; Théry & Casas, 2002; Mäthger *et al.*, 2008; Todd, Lee & Chou, 2009; Zylinksi *et al.*, 2011).

One successful avenue of camouflage research has involved animals that change colour, brightness, and pattern (for simplicity, we refer to 'colour change' in much of this article to encompass these various attributes) to match the background environment (reviewed by Hanlon *et al.*, 2009; Stuart-Fox & Moussalli, 2009). For example, work has investigated what drives camouflage form in cephalopods (e.g. Kelman *et al.*, 2007; Barbosa *et al.*, 2008; Chiao *et al.*, 2011; Zylinski & Johnsen, 2011), chameleons (e.g. Stuart-Fox, Moussalli & Whiting, 2008), teleost marine fish (see Marshall & Johnsen, 2011), and flatfish (e.g. Ramachandran *et al.*, 1996; Kelman, Tiptus & Osorio, 2006). However, colour change and camouflage in animals has predominantly been studied in species that change appearance relatively rapidly (in seconds), despite the fact that these species are restricted to relatively few animal groups. In contrast, numerous species, especially many crustaceans, are reported to change colour (including for camouflage) over longer time periods, such as over several minutes, hours, days, or weeks. For example, the chameleon prawn (*Hippolyte varians*) has been shown to change colour with regards to different substrate types over a period of days to weeks (Keeble & Gamble, 1900). The capacity for less rapid colour change is also found in other animal groups, such as fish (e.g. Clarke & Schluter, 2011).

Comparatively slow changes in camouflage may be widespread in nature and an important method of tuning to the prevailing conditions; however, little work has investigated or quantified this. In addition, studies have focused on the mechanisms underlying colour change rather than the function. The functional basis of slow colour change, and how it influences camouflage, differs from rapid colour change: potentially being found in taxa with different life-history attributes. For example, rapid colour change often occurs in species that are highly mobile or are found against heterogeneous environments (for example cuttlefish), whereas slower colour change may manifest in species that are found in habitats with less variation and that are more predictable, and in species that disperse to different (but stable) background types, such as at during larval stages. Many crabs fit these criteria, and our aim here was to determine the presence of colour change and quantify how this affects camouflage in the horned ghost crab (*Ocypode ceratophthalmus*).

It has been known for a long time that some species of crab have colour changes that occur via a circadian rhythm over night and day (e.g. Atkins, 1926; Abramowitz, 1937). The majority of research has been conducted on a few species of fiddler crab (*Uca* spp., especially *Uca pugilator*), and has shown that the day-to-night rhythm persists for several weeks even when crabs are kept in permanent darkness (Abramowitz, 1937; Brown & Sandeen, 1948; Brown & Webb, 1948; Fingerman, 1956; Fingerman & Yamamoto, 1967; Webb & Lewis, 1977; Darnell, 2012). In most species studied, crabs become darker during the day and lighter at night, as a result of the dispersion and contraction of black and white chromatophore pigments (Abramowitz, 1937; Fingerman, 1955, 1956; Fingerman & Yamamoto, 1967; Rao, Fingerman & Bartell, 1967; Darnell, 2012). These daily cycles continue in the short term, but decline in the longer term without visual moderation (Fingerman & Yamamoto, 1967). Generally, rhythms of colour change in fiddler and other crabs are controlled by hormones of neurosecretory origin, including from the eyestalk (Abramowitz, 1937; Webb & Lewis, 1977; Lacerda & McNamara, 1983; Reddy & Fingerman, 1995; Granato *et al.*, 2004). Frequently, superimposed on top of the daily rhythm are tidal and lunar rhythms of colour change (Fingerman, 1956).

The work briefly reviewed here has focused largely on the mechanisms underlying colour change (e.g. hormonal control and pigment dispersion), and few studies have investigated the adaptive value of this change or its ecological relevance. Furthermore, colour change in *Uca* and other crabs has usually been measured with a 'pigment dispersion index', rather than by quantifying the actual change in coloration (but see Hemmi *et al.*, 2006; Detto, Hemmi & Backwell, 2008), and only Darnell (2012) has analysed the spectral reflectance of a daily rhythm of colour change in crabs. Recent work analysing the circadian rhythm in *Uca panacea*, which also becomes dark during the day and lighter at night, suggested that this mechanism might offer protection from intense ultraviolet light when the crabs are foraging in the open during the day (Darnell, 2012; also see the Discussion). Camouflage has rarely been explored, however, despite the fact that many crab species apparently rely on camouflage against visually hunting predators such as birds (e.g. *Carcinus maenas*; Crothers, 1966; Todd *et al.*, 2005, 2006). At least some species of fiddler crab also show adaptation in coloration with regards to the substrate that they are on. For example, when on black backgrounds *U. pugilator* individuals have more dispersed black chromatophore pigments, whereas on white backgrounds the white pigment is more dispersed (Brown & Sandeen, 1948; Rao *et al.*, 1967).

Figure 1. Images of horned ghost crab (*Ocypode ceratophthalmus*) juveniles from Singapore (left images), from Sabah, Borneo (centre image), and Hainan Island, China (right), showing their camouflage.

In this study, we conduct analyses and experiments on the coloration of *O. ceratophthalmus* from Singapore. *Ocypode* and *Uca* are closely related (both from the family Ocypodidae), and to human eyes *O. ceratophthalmus* juveniles are exceptionally well camouflaged to the beach substrate (Fig. 1), with both the beach and the crabs being light in coloration (i.e. different to the species of *Uca* studied for colour change, which tend to be dark during the day). Background matching is likely to be very important because *O. ceratophthalmus* crabs are exposed from their burrows when foraging and when renewing water in their gill chambers (Cott, 1929; Hughes, 1966). Our aims were first to test for evidence of colour change and the nature of this change (e.g. circadian rhythm and adaptation to match the background), and second to determine whether and how the colour change mediates camouflage in juveniles.

MATERIALS AND METHODS

We collected 18 juvenile crabs on 17 June (J1–J8), 18 June (J9–J10), and 19 June (J11–J18) 2012, from Tanah Merah (1°31′N, 103°97′E) and East Coast Park (1°31′N, 103°94′E) beaches in Singapore. The crabs were collected by capturing them directly on the beach while they were active or by digging them out from their burrows. The crabs were then taken back to the Department of Biological Sciences at the National University of Singapore (NUS), along with seawater and sand from the beaches where they were collected. Individuals were kept under Arcadia Marine White light (FMW 36, 48-inch bulb, Arcadia Products plc, UK) under a lighting regime in the aquarium that followed that of natural day lengths, with lights gradually coming on at sunrise (approximately 0700 h) and gradually turning off at sunset (approximately 1900 h). Crabs were kept on dry sand in individual plastic containers to prevent cannibalism or agonism. Individuals were given periods of time with seawater to wash their gills when not taking part in experiments, and when there were gaps of more than 2 h between measurements.

We only investigated the coloration of juvenile *O. ceratophthalmus*, which seem to be more effectively camouflaged than adults (MS, CPR and PAT, pers. observ.). We classified individuals as juveniles based on an examination of specimens from the Raffles Museum of Biodiversity Research at NUS, collected from various locations in Singapore. Sexual maturity was determined by examining the level of the development of the reproductive organs. The 30 crabs examined were then grouped into three categories and their carapace length measured in relation to their maturity. We determined the carapace widths of: juveniles, < 26 mm; subadults, 26–40 mm; and adults, > 40 mm.

Individuals J1–J10 were used in experiment 1 (day–night cycle analysis), J1–J8 and J11–J17 were used in the darkness experiment, and J1–J4, J6, J8–J18 were used in the substrate experiment. Therefore, even though some crabs were used for more than one experiment (especially J1–J4 and J6), each experiment used individuals that had recently been collected (within 1–2 days). All experiments and measurements were undertaken between 18 and 21 June 2012.

Colour and brightness quantification

We analysed the coloration of the crabs and their camouflage against the beach substrates with digital image analysis, which provides a powerful and non-invasive approach to quantifying animal coloration (Stevens *et al*., 2007; Stevens, Stoddard & Higham, 2009). At the beach where the crabs were collected, we took digital photographs of the sand with a Nikon

D90 digital camera, which had undergone a quartz conversion to provide ultraviolet (UV) light sensitivity (Advanced Camera Services, Norfolk, UK), fitted with a Nikon 105-mm Nikkor lens. For the human-visible photographs (400–700 nm), the lens was fitted with a UV and infrared (IR) blocking filter (Baader UV/IR Cut filter), and for the UV photographs the lens was fitted with a UV pass filter (Baader U filter; 300–400 nm). Each image included a Spectralon grey reflectance standard (Labsphere, Congleton, UK), reflecting light equally at 40% between 300 and 750 nm. We measured 40 different samples of the beach where the crabs were collected from (in situ photographs of the actual beach, both while wet and dry), and analysed the colour and brightness of these samples and compared the results with those collected from the crabs (which were photographed in the laboratory on a small quantity of sand collected from the beach during the three studies; see below). Crabs were photographed individually followed by photographs of the reflectance standard on the same camera settings (the sequential method of calibration; Stevens *et al*., 2009).

Following photography, each image of a crab or background was linearized with regards to light intensity, because many cameras show a nonlinear response in image value with changes in radiance (see Stevens *et al*., 2007). We removed the effects of varying light conditions and scaled each image in terms of the red (longwave; LW), green (mediumwave; MW), and blue (shortwave; SW) layers to reflectance (an image value of 255 on an 8-bit scale equates to 100% reflectance; Stevens *et al*., 2007). Our analyses showed that both crabs and the beach substrate reflected similar levels of UV light to each other. From five samples of the background and from five crabs (photographed during the day), the mean (and standard deviation) UV reflectance values were 18.4% (1.2) and 17.9% (2.0), respectively. In addition, we lacked the necessary equipment (UV light source) to take UV images at night. We also aimed to keep the time for photographing each crab short because previous work with signalling colours in fiddler crabs has shown relatively quick changes in colour in response to stress (Detto *et al*., 2008). Exposure times for UV images are longer, and may have required restraining the crabs; therefore, we only analysed crab coloration in part of the spectrum, from 400 to 700 nm. We are confident, however, that because of the similar levels of UV reflectance in both the sand and the crabs, and the similar levels of UV reflectance to that of the visible spectrum, that our general results are unlikely to be affected by this lack of information.

Following image calibration, we took measurements (one section per crab) of the carapace of the crabs (see below) using the program ImageJ. Measurements were taken to avoid patches of specular reflectance (where light 'bounces' back from the carapace), and were generally taken from the anterior two-thirds of the carapace because the posterior region was often hard to measure depending on the angle that the crab was sitting at. Otherwise, measurements covered as much of the carapace as possible. Note that not measuring the anteriormost parts of the carapace probably makes our analysis conservative, as subjectively the posterior section appeared to change more from day to night. Crabs were photographed quickly (approximately 2 min or less per individual) in order to prevent stress leading to colour change (Detto *et al*., 2008).

The image data were used to calculate several metrics. Overall reflectance (brightness) was calculated as (LW + MW + SW)/3. This is a measure of the overall brightness across the visible spectrum. Perceptually, animals often have luminance or achromatic mechanisms based on MW–LW light. However, if we calculate brightness as LW + MW reflectance or reflectance in the MW part of the spectrum alone, then we obtain R^2 values of 96 and 98%, respectively. Therefore, our results would be unchanged using any of these brightness measures. Colour (or hue) was (LW + MW)/SW. This is a measure of yellow (LW + MW) versus blue (SW) light, and is a ratio analogous to an opponent colour channel (see Spottiswoode & Stevens, 2011; Stevens, 2011). Values above 1.00 mean that the crab or substrate is yellow in colour, values of 1.00 mean that the object is grey, and values less than 1.00 mean that the object is blue. For the colour change analysis over the day–night cycle, we also analysed how much the brightness change of the crabs affected their match to the beach substrate. We calculated a brightness match, being the average difference in brightness between each crab and the 40 different beach substrate samples. Values closer to zero indicate a good match. We also calculated colour camouflage, being the average Euclidian distance in a trichromatic reflectance colour space between each crab and every beach sample. Here, the LW, MW, and SW reflectance values are first standardized to relative proportions to remove absolute variation (Endler & Mielke, 2005), and are then converted into a trichromatic colour space with each point represented by an x and y coordinate. Low Euclidian distance values between the crab and background indicate a close colour match. See Kelber, Vorobyev & Osorio (2003) and Stevens *et al*., (2009) for equations and information on colour spaces. All calibrations and analyses were undertaken with self-written programs in MATLAB (The MathWorks, Inc., MA, USA), and its associated Image Processing toolbox and ImageJ. Ideally, we would like to have analysed crab coloration in terms of predator vision

rather than reflectance. However, the main predators of these crabs are not well documented, and for our initial work we wanted to quantify coloration and background matching objectively, making minimal assumptions about predator vision. Likely predators include birds (with UV vision) and primates such as crab-eating macaques (*Macaca fascicularis*), which have different visual systems (see Discussion). Therefore, for this study we simply analysed changes in overall reflectance (brightness) and in reflectance in different parts of the spectrum (colour).

Day–night cycle

To determine whether the crabs changed colour over the course of a day–night cycle, we photographed ten individual crabs for 48 h at regular time intervals. The lighting varied over the 48 h as a result of natural illumination changes over course of the day and the lighting regime under which the crabs were kept (see above). Individuals J1–J8 were photographed at 0600, 0800, 1200, 1800, 2000, and 0000 h for 2 days, and individuals J9 and J10 were photographed at 1800, 2000, 0000, 0600, 0800, and 1200 h for 2 days. We analysed change in brightness, colour, brightness match, and colour match over time of day with general linear models (GLMs), with individual as a random factor (with six measures per individual for each of the two days).

Darkness experiment

We wanted to determine whether any change in colour or brightness resulted from a day–night (circadian) cycle, or simply from the crabs becoming darker when the light levels were low. We used 15 individuals (J1–J8 and J11–J17). We first measured them after being on dry sand in morning light conditions (0900 h). Then we placed each crab in a plastic container and wrapped each one separately in several layers of dark felt, which excluded all ambient light. We then measured individuals 1 h (1000 h) and 4 h (1300 h) later, followed by removing the felt and putting crabs back into light, with further measurements of individuals after 1 h (1400 h) and after 4 h (1700 h) in the light. For the photographs after 1 h in the dark, we briefly removed the felt before replacing it again for the following 3 h. Our experiment was designed to investigate the following prediction: if crabs change colour in response to dark conditions, then they should get darker under low light levels (especially after 4 h of darkness), whereas if they show a day–night cycle then they should either stay the same colour or become lighter during the dark treatment because these measurements were taken in the middle of the day. Unfortunately, two crabs (J5 and J7) died just before the end of the experiment (just prior to the 4 h light measurement), and therefore these two measurements are missing data points. We used GLMs to test for differences in brightness and colour across the five measurement periods, with individual as random factor (and with five measurements per individual).

Substrate experiment

In our final experiment, we tested whether crabs show changes in colour or brightness when placed on different substrate types. We placed crabs on two different substrates comprising very fine white or black aquarium gravel (2–4 mm; Sudo Phantom, Japan). The LW, MW, and SW reflectance values, and overall brightness values, of the black gravel were 4.6, 4.9, 6.2, and 5.2%, respectively. For the white gravel, these values were 63.8, 62.2, 59.9, and 61.9%. We used 16 individuals (J1–J4, J6, J8–18) that were placed on either white gravel first ($N = 8$) or black gravel first ($N = 8$) and photographed after 4 h (at 1200 h). Following this, individuals were given a 2-h rest period in their individual plastic containers with sand, before being placed on the opposite background, and then photographed after another 4 h (at 1800 h). The crabs were approximately size-matched to the two groups, and placed in positions that controlled for differences in the light levels falling on different containers from windows. The split design of this experiment controlled for changes in colour or brightness during the day as a result of the day–night cycle, as equal numbers of crabs were on each background for the two measurement times. Results were analysed with Wilcoxon–Mann Whitney signed-ranks matched-pairs tests for changes in brightness and colour.

RESULTS

Day–night cycle

Brightness

There was a significant difference in the brightness of crabs among time periods ($F_{5,119} = 16.08$, $P < 0.001$; Fig. 2A), and among individuals ($F_{9,119} = 5.26$, $P < 0.001$). Unplanned Bonferroni pairwise tests showed significant differences between 0600 and 0800 h ($T = 3.37$, $P = 0.016$), between 0600 and 1200 h ($T = 4.78$, $P < 0.001$), between 0800 and 2000 h ($T = 5.73$, $P < 0.001$), between 0800 and 0000 h ($T = 5.25$, $P < 0.001$), between 1200 and 1800 h ($T = 3.84$, $P = 0.003$), between 1200 and 2000 h ($T = 7.13$, $P < 0.001$), and between 1200 and 0000 h ($T = 6.65$, $P < 0.001$). Overall, crabs became darker during the night, and lighter during the day.

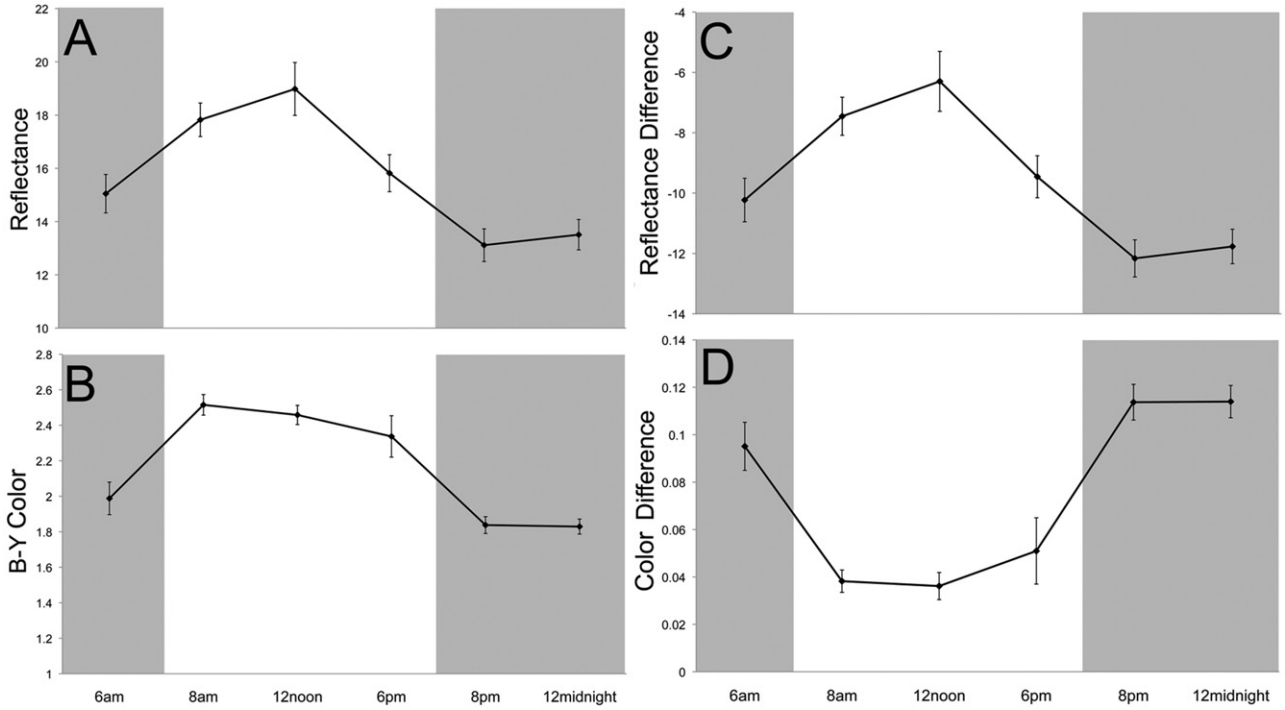

Figure 2. Changes in brightness (A, overall reflectance) and blue–yellow colour (B) across time periods, with SE bars. Crabs get significantly lighter and become significantly more yellow and less blue/grey during the day. Graphs on the right show that the level of camouflage against the sand is significantly better during the day for both brightness (C, closer to 0% reflectance difference) and colour (D, lower colour difference values).

Colour

There were significant differences in the colour of crabs among time periods ($F_{5,119} = 44.92$, $P < 0.001$; Fig. 2B) and among individuals ($F_{9,119} = 8.52$, $P < 0.001$). Unplanned Bonferroni pairwise tests showed significant differences between 0600 and 0800 h ($T = 7.99$, $P < 0.001$), between 0600 and 1200 h ($T = 7.13$, $P < 0.001$), between 0600 and 1800 h ($T = 5.29$, $P < 0.001$), between 0800 and 2000 h ($T = 10.26$, $P < 0.001$), between 0800 and 0000 h ($T = 10.39$, $P < 0.001$), between 1200 and 2000 h ($T = 9.40$, $P < 0.001$), between 1200 and 0000 h ($T = 9.53$, $P < 0.001$), between 1800 and 2000 h ($T = 7.56$, $P < 0.001$), and between 1800 and 0000 h ($T = 7.69$, $P < 0.001$). Overall, crabs became more yellow during the day, and became more grey (i.e. with a greater relative proportion of blue) during the night.

Crabs therefore showed clear day–night cycles. Even though the average changes across individuals for colour and brightness at any given time point were quite small, there was substantial variation among different individuals (Fig. 3; Figs S1 and S2), and the magnitude of change on an individual level across time periods was often quite high. Some crabs showed very strong day–night rhythms, whereas others had less defined changes. In most cases, the intra-individual range in terms of maximum difference in brightness across all time periods was about 10%, and for several individuals it was closer to 20%. For colour, the maximum differences on an individual level were usually just above 1.00, but could be close to 2.00. These changes were clearly perceptible to the human eye.

Brightness matching

There were significant differences in the brightness of crabs against the background among time periods ($F_{5,119} = 16.08$, $P < 0.001$; Fig. 2C) and among individuals ($F_{9,119} = 5.26$, $P < 0.001$). Unplanned Bonferroni pairwise tests showed significant differences between 0600 and 0800 h ($T = 3.37$, $P = 0.015$), between 0600 and 1200 h ($T = 4.78$, $P < 0.001$), between 0800 and 2000 h ($T = 5.73$, $P < 0.001$), between 0800 and 0000 h ($T = 5.25$, $P < 0.001$), between 1200 and 1800 h ($T = 3.84$, $P = 0.003$), between 1200 and 2000 h ($T = 7.13$, $P < 0.001$), between 1200 and 0000 h ($T = 6.65$, $P < 0.001$), and between 1800 and 2000 h ($T = 3.29$, $P = 0.021$). Therefore, crabs deviated less in brightness from the beach background during daylight hours.

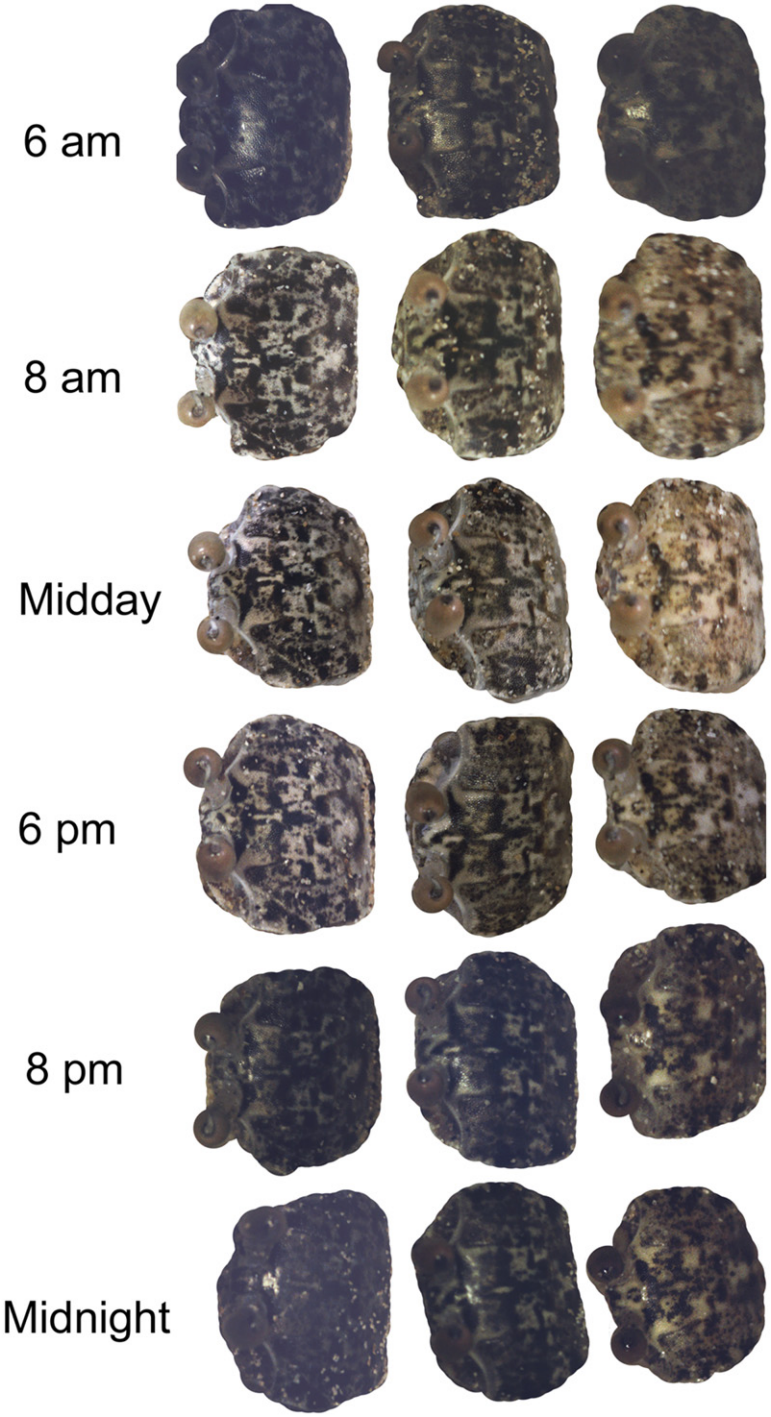

Figure 3. Variation in colour and brightness in three crabs over the 24-h cycle. Images are reflectance data, but all images have been increased in brightness by 80 pixel values to make viewing easier.

Colour matching

There were significant differences in colour against the background among time periods ($F_{5,119} = 53.19$, $P < 0.001$; Fig. 2D) and among individuals ($F_{9,119} = 9.94$, $P < 0.001$). Unplanned Bonferroni pairwise tests showed significant differences between 0600 and 0800 h ($T = 7.92$, $P < 0.001$), between 0600 and 1200 h ($T = 8.21$, $P < 0.001$), between 0600 and 1800 h ($T = 6.15$, $P < 0.001$), between 0800 and 2000 h ($T = 10.51$, $P < 0.001$), between 0800 and 0000 h

($T = 10.55$, $P < 0.001$), between 1200 and 2000 h ($T = 10.80$, $P < 0.001$), between 1200 and 0000 h ($T = 10.83$, $P < 0.001$), between 1800 and 2000 h ($T = 8.74$, $P < 0.001$), and between 1800 and 0000 h ($T = 8.77$, $P < 0.001$). The change in colour during the day, becoming more yellow, means that crabs became significantly closer in coloration to the sand during the day (Fig. S4).

Darkness experiment

Brightness

There were significant differences in the brightness of crabs among time periods ($F_{4,72} = 6.04$, $P < 0.001$; Fig. 4) and among individuals ($F_{14,72} = 6.58$, $P < 0.001$). Unplanned Bonferroni pairwise tests showed significant differences between the start and 4 h of dark ($T = 3.44$, $P = 0.011$), between 1 h of light and 4 h of dark ($T = 3.06$, $P = 0.034$), between 4 h of light and 4 h of dark ($T = 4.27$, $P < 0.001$), and between 1 h of dark and 4 h of light ($T = 3.02$, $P = 0.038$). Contrary to the prediction that crabs become dark when under low light levels, crabs actually became significantly lighter during the darkness phase of the experiment. This coincides with the middle of the day, when crabs may be at their lightest because of a circadian day–night cycle.

Colour

There were significant differences in the colour of crabs among time periods ($F_{4,72} = 4.28$, $P = 0.004$; Fig. 4) and among individuals ($F_{14,72} = 25.10$, $P < 0.001$). Unplanned Bonferroni pairwise tests showed significant differences only between the start and 4 h of dark ($T = 3.96$, $P = 0.002$). Contrary to the hypothesis that darkness mediates colour change, crabs became yellower during the darkness phase of the experiment, which coincides with the middle of the day.

Substrate experiment

Brightness

There was a significant difference in individual crab brightness after the period spent on white gravel

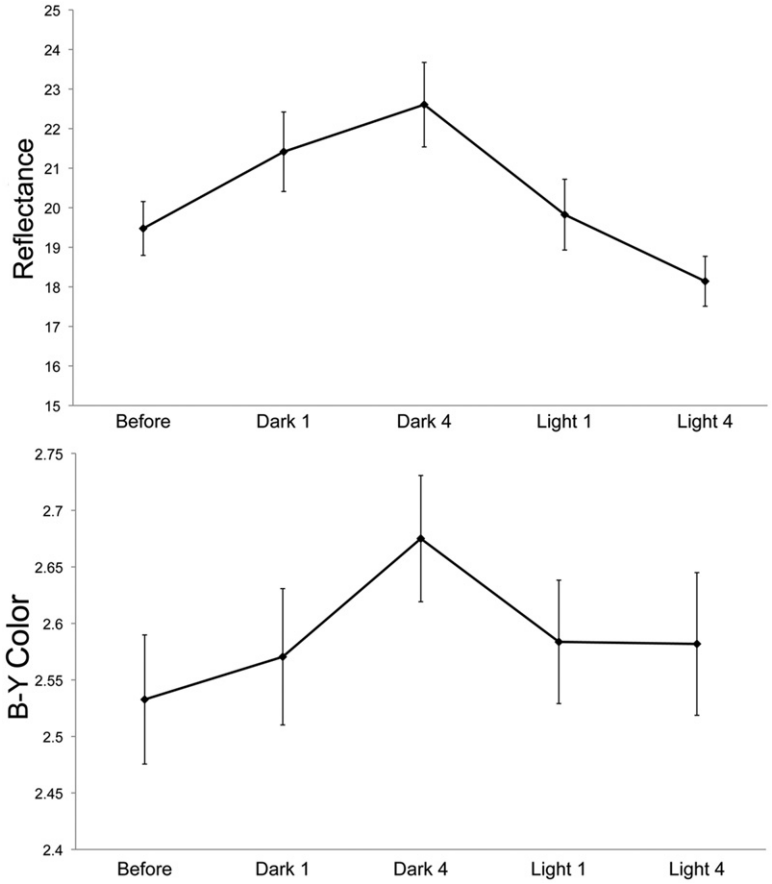

Figure 4. Changes in the brightness (overall reflectance; top) and blue–yellow colour (bottom) of crabs when placed under dark conditions and then back into the light. The crabs became lighter and yellower when in the dark, probably because this coincided with the middle of the day and the peak colour change in their day–night cycle.

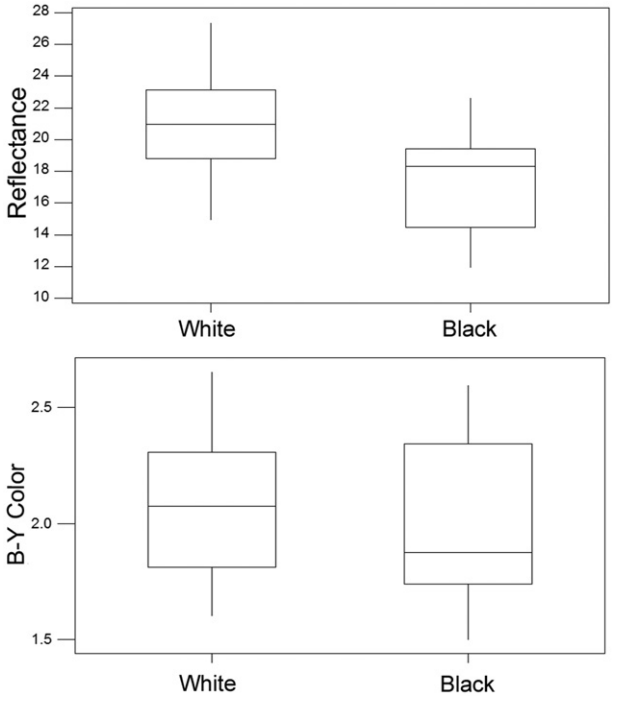

Figure 5. Changes in brightness (overall reflectance; top) and blue–yellow colour (bottom) when crabs were placed on either black or white substrates. Crabs became lighter but did not change colour when put onto a white versus a black background.

compared with black gravel, with crabs becoming brighter on white gravel ($W = 117.00$, $N = 16$, $P = 0.012$; Fig. 5; Fig. S3). Again, even though the average change in brightness across individuals was relatively small, there was much individual variation. Some individuals changed very little, whereas seven of the individuals became between 6 and 10% lighter on the white substrate. Some variation in response may have come from changes associated with the circadian rhythm. For example, the four individuals that became darker on the white substrate were photographed on the black substrate at midday and the white substrate at 1800 h (i.e. towards the end of the day).

Colour

There were no significant differences in individual crab colours after the period spent on white gravel compared with black gravel ($W = 86.00$, $N = 16$, $P = 0.366$; Fig. 5).

DISCUSSION

Here, we have demonstrated that juvenile *O. ceratophthalmus* show a daily rhythm of colour change, becoming darker at night and lighter and more yellow during the day. We have also demonstrated that they show a brightness change in response to the substrate, becoming lighter on a white substrate than on a black one. These changes improve the level of matching with the sand substrate upon which the crabs are found.

Even though the average level of change across all individuals at any given time point was relatively small (Figs 2 and 5), changes across time periods at an individual level were often quite large, and were clearly perceptible to human eyes (Fig. 3; Figs S1–S3). Based on previous findings in some species of *Uca*, the large variation among individuals could be explained by the location of individuals with respect to high tide level and lunar cycles. Activity and patterns of colour change in the tidal cycle of *Uca* species varies across individuals depending on the height of the burrow on the beach, because the time and duration that burrows are covered by water depends on their location (Fingerman, 1956). Individuals with burrows above the high tide mark seem to lack tidal rhythms, as they can leave their burrows at any time, whereas individuals below this have to remain in their burrows until the tide has left (Fingerman, Lowe & Mobberly, 1958). We also acknowledge that measurement error may lead to some imprecision in our measurements because the crabs were not immobilized (to reduce stress), and so their body posture was hard to control. This meant that the exact area of the crab measured was not always consistent. Nevertheless, the overall variation observed among individuals does not seem unusual given the variation that is known to exist in *Uca* species.

In the darkness experiments, where crabs were placed under dark conditions for several hours, the changes in brightness were opposite to that predicted. Individuals became significantly lighter and yellower during the dark period, and then darker again afterwards. This is consistent with changes in coloration occurring with a day–night cycle: crabs were lightest/most yellow after 4 h in the dark, corresponding to 1300 h (i.e. just after midday). The average values for individuals in the dark were similar to those for the circadian rhythm experiment at a similar time point. In fact, they are slightly higher, although this could be because some different individuals were used and because the measurement was an hour later in the darkness experiment than for the circadian analysis. Therefore, our results strongly suggest that the ghost crabs undergo a change in coloration that is driven by a circadian rhythm, rather than being a direct response to ambient light levels (but the timing of light conditions should modulate a daily rhythm over time, and we cannot rule out a change that occurs in response to a lack of visual input). This finding makes sense because crabs should be under selection not to

change colour when they enter the darkness of their burrows. Otherwise, when they emerge in the day they would be conspicuous as a dark object against the light-yellow sand.

Crabs also became significantly brighter when placed on a white substrate than when they were placed on black gravel. This indicates that when they can see the substrate (i.e. not just being in darkness or light) individuals can fine-tune their camouflage to the brightness of the substrate, over and above their day–night cycle. In our experiments the crabs did not change colour, only brightness. However, both backgrounds used were achromatic, and so we cannot determine whether individuals would be able to change colour when placed on substrates that differ in colour. In the chameleon prawn, Keeble & Gamble (1900) suggested that adaptation to the local background was influenced by the ratio of incident light to reflected light: on a black background the ratio is larger, whereas on a white background the ratio is smaller, and this could allow individuals to change to match the brightness of the background rather than simply to the light conditions. This relationship may also mediate the camouflage response in flatfish (Sumner & Keys, 1929), and changes in crabs and other species could be brought about by light falling on different parts of the eye (Brown & Sandeen, 1948).

We could not control the ambient temperature during our study, and so cannot dismiss an influence of temperature on colour change. However, ambient temperature is unlikely to be the primary driving force of the daily colour change. In at least some fiddler crabs and other species, the daily, tidal, and lunar rhythms are largely independent of temperature changes, or temperature has a relatively weak influence on modifying the circadian cycles (Darnell, 2012), and crabs show their characteristic changes over a wide temperature range (e.g. 6–26 °C; Brown & Webb, 1948; Brown *et al*., 1954; Fingerman, 1955, 1956; Fingerman & Yamamoto, 1967). Also, our results for adaptation to the background brightness cannot be an effect of temperature because the design of our experiment involved half of the crabs being held on the dark substrate and half being held on the white substrate for each trial period. There can, however, be direct responses of the chromatophores to temperature changes (Silbiger & Munguia, 2008), and temperature may mediate adaptation to backgrounds to some degree (Brown & Sandeen, 1948). Therefore, it would be worthwhile to determine what, if any, effect temperature has on colour change in *O. ceratophthalmus*.

Generally, in the species that have been studied previously, fiddler crabs become dark during the day and light in colour at night, as a result of the concentration of black pigment at night and a dispersal of this pigment during the day (Brown & Webb, 1948; Darnell, 2012). Brown & Sandeen (1948) suggest that in *U. pugilator* the dark diurnal coloration may have a thermoregulatory function or protect individuals from damage from ultraviolet light. Protection from UV has also been suggested for the change to dark coloration during the day in *U. panacea* (Darnell, 2012), but there is a key difference between these species of fiddler crab and the *O. ceratophthalmus* of our study: *O. ceratophthalmus* occur in tropical environments with intense UV radiation during the day, yet they become lighter during the day and dark at night (the opposite of *Uca* above). It is possible that being light during the day enables crabs to stay cool more effectively. We have, however, demonstrated that the change in appearance significantly improves the degree of camouflage during the day to the light-yellow sandy substrate. Furthermore, any adaptation to light and dark backgrounds would serve to fine-tune the degree of matching to different beach types. Therefore, in contrast to *Uca*, both the daily rhythm and the background adaptation response in *O. ceratophthalmus* appear to have a camouflage function.

One issue that is currently unclear is why crabs become dark at night, rather than simply maintaining their level of matching with the background. At first this seems odd because, even though the level of light intensity is much lower at night, the level of contrast between the crab and background would be unchanged. We suggest that changing to a dark coloration may nonetheless still afford effective camouflage. Colour vision stops functioning at low light levels, and although the light levels for which this happens is poorly known for many species it is likely that, especially for animals with good colour vision and multiple cone types, such as birds, colour vision would be ineffective under moonlight conditions. This means that matching the colour of the background is not needed at night. The reason for a change in brightness is harder to determine and two possibilities exist. One is that detection probability is low and so crabs can afford a loss of camouflage if this is offset by other benefits, perhaps in thermoregulation. Alternatively, and the explanation that we favour, is that the crabs are switching to a strategy of matching the dark shadows that fall on the beach at night. Predation pressure from wading birds and other species is likely to be high, both during the day and at night, because shore birds will forage whenever the tide is out. The mechanisms are not well understood, but perceptually shadows (such as between ridges in the sand and from vegetation above) often appear more pronounced and of greater contrast at night than during the day. This may occur if the overall contrast sensitivity to shadows is higher under lower absolute

illumination levels. In addition, the moon will often be relatively low in the sky, providing further elongated shadows falling on the beach from vegetation. Therefore, crabs may be dark in order to blend into these shadows and to mimic them when walking on shadowless patches of moonlit beach.

Overall, to our knowledge this is the first study to quantify changes in coloration under a daily rhythm with respect to the background appearance, and showing that camouflage can be mediated by a day–night cycle. However, our experiments leave a number of issues unresolved. First, we did not model camouflage to predator vision. This was partly because the main predators are not well documented and so we selected objective measurements instead (reflectance), but also because we chose to refrain from physically restraining the crabs, thereby making it difficult to take the ultraviolet photographs that would be needed to model predator vision. In future, it would be valuable to model camouflage to predator vision, and to determine how colour change influences the matching of the crabs' two-dimensional pattern with the background. Birds and mammals are both likely predators (Cott, 1929; Jones, 1972; Hughes, 1966). For example, the common myna (*Acridotheres tristis*) has been observed eating *O. ceratophthalmus* in Singapore (N. K. Ng, pers. comm.). Other local predators may include osprey (*Pandion haliaetus*), black-winged kite (*Elanus caeruleus*), Brahminy kite (*Haliastur indus*), white-bellied sea eagle (*Haliaeetus leucogaster*), smooth-coated otter (*Lutrogale perspicillata*), common palm civet (*Paradoxurus hermaphroditus*), and the crab-eating (long-tailed) macaque (*Macaca fascicularis*). We have also observed the collared kingfisher (*Todirhamphus chloris*) and *H. indus* hunting on beaches where *O. ceratophthalmus* is found in Sabah, Borneo, and have seen various wading birds in Hainan Island, China, on beaches hosting *O. ceratophthalmus*. Thus, the most common predators of crabs are probably birds, but with mammals also contributing to predation, thereby representing a range of visual systems. Studies also need to demonstrate a change in predator detection responses with colour change.

Finally, we note that the level of camouflage can be highly refined in crabs living in some locations, such as individuals in Borneo (Fig. 1), including apparently matching fine-scale colours and features of specific beach types that seems unlikely to be driven by changes in coloration of the type observed in this study. It would be valuable to determine what degree of background matching is driven by changes in coloration, and what is genetically controlled between populations. More investigations of the many species of animal that may show colour change over similar time periods would help to determine how camouflage appearance is tuned to environmental features. As an area of study, animal camouflage has much to tell us about the mechanistic basis of how animals adapt to different environments (Nachman *et al*., 2003; Manceau *et al*., 2011), how selection pressure from predators can drive inter- and intraspecific variation and divergence (e.g. Nosil & Crespi, 2006; Clarke & Schluter, 2011; Pellissier *et al*., 2011), and the relationship between animal coloration and visual perception (Osorio & Srinivasan, 1991; Stevens & Cuthill, 2006; Troscianko *et al*., 2009; Zylinski *et al*., 2009). Studies of a wider range of real animals against their natural habitats will be important in this field.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher's website:

Figure S1. Changes in brightness (overall reflectance) of ten individual crabs over the course of 48 h, illustrating the level of variation that exists among individuals. Individuals 9 and 10 were collected later, and the start and end of their 48-h measurement period differed from that of the other individuals.

Figure S2. Changes in the blue–yellow colour of ten individual crabs over the course of 48 h, illustrating the variation that exists among individuals. Individuals 9 and 10 were collected later, and the start and end of their 48-h measurement period differed from that of the other individuals.

Figure S3. Individual differences in brightness for individuals on the black and white substrates. Four individuals (dashed lines) became slightly darker on the white substrate, whereas 12 individuals became lighter on the white substrate (solid lines). All four individuals that became darker on the white substrate were placed on the white substrate second, and were photographed on the black substrate at midday and the white substrate at 1800 h. It is therefore possible that these individuals became darker if they were changing to their night-time appearance.

Figure S4. A trichromatic reflectance-based colour space plot based on longwave (LW), mediumwave (MW), and shortwave (SW) reflectance values converted to (x, y) coordinates, comparing individual crabs photographed at 1200 h (red symbols) and 0000 h (blue symbols), versus the samples of the sand background colour from the beach where the crabs were collected. Crabs became more yellow, like sand, during the day.